A Phase I Study of Active Immunotherapy with Autologous Dendritic Cells Infected with CEA-6D Expressing Fowlpox-Tricom in Patients with Advanced or Metastatic Malignancies Expressing CEA

SCIENTIFIC ABSTRACT

This proposal is based upon the premise that a clinically effective cell mediated immune response against "non-immunogenic" spontaneously arising tumors can be elicited by activation of tumor associated antigen (TAA) specific cytotoxic T cells (CTL). Carcinoembryonic antigen (CEA) is an oncofetal protein, overexpressed in most gastrointestinal cancers and approximately 50% of lung cancer and 50% of breast cancers. CEA may serve as a CTL target and demonstration of protective immunity to CEA in CEA expressing murine tumors has been reported by Schlom and colleagues.

Schlom and colleagues have demonstrated human anti-CEA CTL can be induced in patients following vaccination with recombinant vaccinia virus expressing CEA and have defined CTL epitopes to CEA such as the HLA A2 binding nanomer CAP-1. Multiple vaccinations using vaccinia vectors are limited by the neutralizing immune response against vaccinia, therefore alternative vaccine strategies need to be explored. Attempts to immunize patients with autologous cultured dendritic cells (DC) pulsed with peptide epitopes of CEA, specifically CAP-1 have been shown to be safe, but elicited minimal immune responses. Attempts to immunize patients with DC modified to express CEA (by loading with CEA mRNA) have been shown to be safe.

Two important advances in DC immunotherapy have been made. First, a modified CAP-1 peptide has been constructed by Schlom and colleagues, which replaces a valine at the 6^{th} amino acid, increasing the immunogenicity of the peptide. Second, analysis of the DC administered in our previous clinical levels demonstrate unexpectedly low expression of B7.1 (CD80), and ICAM (CD54). These could be increased by infecting DC with fowlpox vectors expressing B7.1, ICAM, and LFA3 (heretofore referred to as the triad of costimulatory molecules "TRICOM"). Schlom's group has developed a fowlpox vector containing the TRICOM construct and the gene for CEA (rF-CEA(6D)/TRICOM). We have demonstrated that rF-CEA(6D)/TRICOM modified DC (whether fresh or previously cryopreserved) have increased ability to induce cytotoxic T cells *in vitro*. We therefore propose to test an immunization strategy utilizing autologous DC modified with rF-CEA(6D)/TRICOM to determine if CEA -specific T cells will be induced *in vivo.*

In order to evaluate the immune response against CEA, it is important to use control antigens as well as comparators. We have previously used tetanus toxoid mixed with DC and found this to induce potent T cell-mediated immune responses in the injected cancer patients. We have also demonstrated that DC loaded with the HLA A2 restricted CMV pp65 peptide induces CMV specific T cell responses. Thus, both antigens could serve as control vaccines.

The specific objectives of this proposal are:

a) The primary objective of this protocol is to determine the safety and feasibility of one, two, or three cycles of TRICOM modified DC expressing full length CEA-6D, where one cycle of immunotherapy consists of a leukapheresis to obtain peripheral blood DC

precursors and *in vitro* generation of the DC from these precursors followed by 1 injection every 3 weeks for a total of 4 injections of TRICOM modified DC expressing full length CEA-6D.

b) As a secondary objective, the immune response of patients with TRICOM modified DC expressing full length CEA-6D over expressing malignancies to the intra-dermal injection of TRICOM modified DC expressing full length CEA-6D will be evaluated.

c) A third objective will be to collect preliminary data on response rate.